

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

whereby one offers the bacteria at least one substrate carbon source which is selected from the group consisting of: levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyheptanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures; one incubates the bacteria for a certain time with the carbon source; and one isolates the poly(hydroxy fatty acid) polymers that have been synthesized by the bacteria, it has been possible for the first time to produce 4HV-containing polyesters starting out from levulinic acid. The chemical structure of levulinic acid is reproduced in the following formula:--

In the Claims

1. (Amended) [Process] A process for the preparation of poly(hydroxy fatty acids) comprising incubating a recombinant organism in a mineral medium under aerobic conditions, expressing [with at least one subunit by means of recombinant bacteria which contain and express] at least one fragment of the gene [of] encoding the poly(hydroxy fatty acid) synthase from *Thiocapsa pfennigii* with a substrate carbon source, wherein the recombinant organism produces a poly(hydroxy fatty acid) and,

the poly (hydroxy fatty acid) is recovered [and which are selected from the group comprising:

Pseudomonas putida GPp104 (pHP1014::E156), *Alcaligenes eutrophus* PHB 4 (pHP1014::EIS6), *Pseudomonas putida* GPp104 (pHP1014::B28+) [DSM #9417] and *Alcaligenes eutrophus* PHB 4 (pHP1014:B28+) [DSM # 9418], whereby the bacteria are cultivated in a mineral medium under aerobic conditions, whereby

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

• one offers the bacteria at least one substrate carbon source which is selected from the group consisting of:

levulinic acid, salts of levulinic acid, esters of levulinic acid, lactones of levulinic acid, substituted levulinic acid or, as the case may be, its derivatives; 5-hydroxyhexanoic acid, its salts, esters and lactones; 4-hydroxyoctanoic acid, its salts, esters and lactones; their halogenated derivatives as well as their mixtures;

• one incubates the bacteria for a certain time with the carbon source; and
• one isolates the poly(hydroxyl fatty acid) polymers that have been synthesized by the bacteria.]

2. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the bacteria are pre-cultivated in a complex medium.

[4] 3. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein one also adds to the bacterial culture at least one additional carbon source which promotes growth, whereby the carbon source is selected from the group [comprising:] consisting of

citric acid, citric acid salts, citric acid esters and citric acid lactones, octanoic acid, octanoic acid salts, octanoic acid esters, octanoic acid lactones, [and] gluconic acid, gluconic acid salts, gluconic acid esters, gluconic acid lactones, [; their salts, esters and lactones;] hexoses, [especially glucose and fructose; as well as their mixtures] and combinations thereof.

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

4. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the process is carried out in the form of a batch process, a fed-batch process, a two-step process or a continuous flow process.

5. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 70% by weight [or, especially approximately 15 to 50% by weight or, preferably, approximately 40% by weight] based on the dry mass of the bacterial cells.

6. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the poly(hydroxy fatty acids) are obtained in the form of copolymers with at least two [or preferably, three] subunits.

7. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the recombinant bacteria are cultivated at cell densities of up to 100 g of dry cellular mass per liter of bacterial nutrient medium.

8. (Amended) [Process in accordance with] The process of [claims] claim 1, [characterized by the feature that] wherein one offers the substrate carbon source in excess.

9. (Amended) [Process in accordance with] The process of claim 8, [characterized by the feature that] wherein one [uses] offers the substrate carbon source at a concentration of approximately 0.1 to 5% by weight.

10. (Amended) [Process in accordance with] The process of claim 9, [characterized by the feature that] wherein one increases the concentration of the substrate carbon source in the

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

culture medium in steps, optionally with pre-cultivation in the presence of an additional carbon source which does not serve as a substrate.

11. (Amended) [Process in accordance with] The process of claim 10, [characterized by the feature that] wherein, [in each case], one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 27°C to 35°C [or, preferably, at approximately 30°C].

12. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein cultivation takes place for approximately 24 h to 96 h [or, especially, for approximately 36 h to 72 h or, preferably, for approximately 48 h to 72 h].

13. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the recombinant bacteria are cultivated under conditions deficient in an element [of deficiency, preferably under] wherein the element is selected from the group consisting of [conditions of a deficiency of] nitrogen, magnesium or phosphate.

14. (Amended) [Process in accordance with] The process of claim 1, [characterized by the feature that] wherein the harvested recombinant bacteria are broken open [by means of physical and/or chemical and/or biochemical processes] in order to obtain the poly(hydroxy fatty acids) that have been produced [bio-technically].

15. (Amended) [Process in accordance with] The process of Claim 14, [characterized by the feature that] wherein the harvested recombinant bacteria are lyophilized and then extracted with an organic solvent, [preferably] selected from the group consisting of

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

chloroform or methylene chloride, in order to break open the recombinant bacteria and to obtain the poly(hydroxy fatty acids).

16. (Amended) [Process in accordance with] The process of claim 15, [characterized by the feature that] wherein the extracted poly(hydroxy fatty acid) produced is precipitated by introducing a hydrophilic solvent, [especially] selected from the group consisting of water [or] and a lower alcohol, [preferably ethanol,] [and] wherein the product is obtained in essentially pure form by removing the hydrophilic solvent.

17. (Amended) [Process in accordance with] The process of claim 14, [characterized by the feature that] wherein the harvested recombinant bacteria are broken open by means [of] selected from the group consisting of detergents, [and/or] a lytic enzyme cocktail, and a combination thereof [as a result of which] wherein the bacterial cell grana, which contain the poly(hydroxy fatty acid), sediment to the bottom of the bio-reactor and are collected from there in order to be processed further.

18. (Amended) [Process in accordance with] The process of claim 17, [characterized by the feature that] wherein the lytic enzyme cocktail contains enzymes which are selected from the group [which comprises:] consisting of

lysozyme; proteases; other hydrolytic enzymes; [as well as their mixtures] and combinations thereof.

Please add the following new claims 37-42.

37. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 15 to 50% by weight based on the dry mass of the bacterial cells.

U.S.S.N. 09/779,427

Filed: February 8, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

38. The process of claim 1, wherein the poly(hydroxy fatty acid) is obtained in a concentration of approximately 40% by weight based on the dry mass of the bacterial cells.

39. The process of claim 10, wherein, in each case, one adds approximately 0.5% (weight/volume) of neutralized substrate carbon source after approximately 12 h and 24 h at approximately 30°C.

40. The process of claim 1, wherein cultivation takes place for approximately 36 h to 72 h.

41. The process of claim 1, wherein cultivation takes place for approximately 48 h to 72 h.

42. The process of claim 1 wherein the poly(hydroxy fatty acid) polymer is comprised of one or more monomers selected from the group consisting of :

- (A) 3-hydroxybutyric acid, 3 hydroxyvaleric acid and 4-hydroxy-valeric acid;
- (B) 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 4-hydroxy-valeric acid, 3-hydroxyhexanoic acid and 3-hydroxyoctanoic acid;
- (C) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid, 5-hydroxyhexanoic acid, and 3-hydroxyoctanoic acid;
- (D) 3-hydroxybutyric acid, 3 hydroxyvaleric acid, 3-hydroxyhexanoic acid, 3-hydroxyheptanoic acid, 4-hydroxyheptanoic acid and 3-hydroxyoctanoic acid;
- (E) 3-hydroxybutyric acid, 3 hydroxyhexanoic acid, 3-hydroxy-octanoic acid and 4-hydroxyoctanoic acid;
- (F) 3-hydroxybutyric acid, 3-hydroxyhexanoic acid and 5-hydroxyhexanoic acid;